

**HOW CAN DIFFERENT RINSING SOLUTIONS INFLUENCE THE
HEALING PROCESS OF THE CORNEA AFTER AN OCULAR BURN ?
ELEMENTARY CONDITIONNING OF THE CORNEA BY
PROLONGED APPLICATION OF EYE DROPS.**

S. Langefeld, W. Aschenbrenner, A. Scherer, M. Reim and N.F. Schrage

AFFILIATIONS :

Universitäts augenlinik der RWTH Aachen
Pauwelstrasse 30
52057 Aachen
GERMANY
Phone : 00 49 241 8088224
Fax : 00 49 241 8888438

Please address any correspondance to :

Dr Stephanie Langefeld
Universitätsaugenlinik der RWTH Aachen
Pauwelstrasse 30
52057 Aachen
GERMANY
Phone : 00 49 241 8088224
Fax : 00 49 241 8888438

Summary :

Aim of the study : After an ocular burn, an efficient treatment by irrigation is crucial for the healing process of the damaged eye. The rinsing solution has to be chosen according to its chemical neutralisation capacity, its biocompatibility and its adjustment to the physiological environment of the cornea. In case of a prolonged treatment by rinsing, the influence of the irrigation solution is particularly important. During this study, we will show that the buffer effect itself is not sufficient.

Material and methods : After being burnt with 1N NaOH during 30 seconds, the eye of 24 rabbits were irrigated either with an 0,9% saline solution, or with a phosphate buffer solution, or with Diphotérine®. The pH of the cornea and the anterior chamber were measured during the first step of the experimentation. During the second stage, the treatment by rinsing was pursued either with a phosphate buffer solution or with 0,9% saline solution applied 3 times a day during 16 days. A clinical valuation was carried out each day during the experimentation. After 16 days, the elementary composition of the cornea was measured thanks to an Energy Dispersion X ray analysis (EDXA).

Results : after 16 days, the cornea of the 8 rabbits treated by irrigation with a phosphate buffer were completely calcified. From a clinical point of view, the opacification of the cornea had already been noted after 2 days of treatment. The EXDA measures confirmed the calcification.

An accumulation of sodium and chloride was noted after irrigation with a 0,9% saline solution. The concentration in sulphur had decreased after both treatments.

Conclusion : The power of phosphate buffer and Diphotérine® is similar. But the irrigation with a phosphate buffer provokes a calcification of the cornea. Clinical observations of ocular burns on patients confirmed these results. The electrolytic modifications noted after both types of irrigation show the chemical power of these solutions on a naked cornea. It is necessary to build up an irrigation solution adapted to the physiology of the corneal stroma. Until now, the irrigation with Diphotérine® seems to have had a beneficial effect. New studies are necessary concerning the long term irrigation with Diphotérine®.

Introduction

Ocular burns due to alkali need an efficient and quick emergency treatment.

The clinical practice shows that the time lapsed between the accident and the first eye rinsing plays an important role in the cornea healing. The dilution of the burning agent by irrigation with tap water is not sufficient. Researches show that buffer solutions have an additional positive effect on the pH development both from the cornea and the anterior chamber¹. Most of the irrigation solutions, for example phosphate buffer, are also used for the prolonged treatment of burnt eyes. The fact that the phosphate buffer solution or another rinsing solution used in clinical practice, for example an isotonic saline blood solution, has a negative effect after a prolonged use on a naked cornea had never been systematically tested before.

It is necessary to take into account the fact that the irrigation is made on a naked cornea which is not able to restore the ionic balance anymore. The effect of a continuous irrigation with a large quantity of electrolytes must be considerable. Furthermore, no effort was made to adapt these solutions to the ionic environment of the corneal stroma as it was done for the blood. This induces important osmotic effects which must in a certain way modify the healing conditions of the cornea.

We researched in this study if the ionic components of these rinsing solutions (phosphate buffer solution, saline solution) have an effect on the healing process of the cornea or not.

Material and methods

Three different rinsing solutions were tested during this study in a double blind way :

1. 0,9% NaCl solution "isotonic" and sterile
2. "isotonic" and sterile phosphate buffer solution
3. Diphotérine[®]/Previn[®]

During the first step the corneal and aqueous pH were measured after a burn with alkali and irrigation with the three above-mentioned solutions. Under deep general anaesthesia , the right eye of 18 rabbits was burnt with 5 ml of 1N NaOH during 30 seconds. The burn was made with a Plexiglas cylinder covering the cornea and 1 mm of the limb. The alkali was removed after 30 seconds. After an immediate rinsing with 150ml of solution during 5 minutes, the pH measure was repeated for the surface of the cornea as well as for the aqueous humour.

In the second stage a study was carried out in a double blind way. The eyes of 24 rabbits were burnt with 1N NaOH during 30 seconds. Immediately after the burn an irrigation was made with 150ml of each of the three solutions mentioned here under during 5 minutes. During the 16 following days both eyes of the 8 rabbits were rinsed three times a day with 160ml as described :

- 8 rabbits with phosphate buffer solution for the first irrigation and the prolonged treatment
- 8 rabbits with an 0,9% saline solution for the first irrigation and the prolonged treatment
- 8 rabbits with Diphotérine[®] for the first irrigation and a saline solution as prolonged treatment

A clinical valuation of the eyes was carried out every day. After 16 days the eyes were enucleated and the cornea frozen in liquid nitrogen between two pre cooled metal blocs. Preparations for REM microscopy and histological valuation were done. The complete animal experimentation was carried out according to Helsinki declaration directives and local laws from the Federal German Republic.

We made an X ray energy dispersion analysis on the frozen tissues in order to determine the elementary composition of the different cornea layers. A quantification was possible after a calibration measure².

Results

pH measures :

The irrigation with 250ml of saline solution does not diminish the pH of the cornea or the aqueous humour very much. Buffer solutions, like phosphate buffer or Diphotérine[®] reduce the pH to 7,5 on the cornea and to 9,5 in the aqueous humour (fig 1 / 2).

After prolonged irrigation, complete calcifications of the cornea were noted in all cases in the group receiving a phosphate buffer irrigation. The opacification appeared on the second day of the irrigation (fig 3). The calcification was confirmed by EDX analysis which showed a high concentration in P and Ca (fig 4). Figure 5 shows a gradient of epithelium calcifications with important accumulations of P/Ca close to the Descemet membrane where no increase in the P and Ca concentration was detected.

As the Diphotérine[®] group underwent a rinsing with an 0,9% saline solution after the first irrigation, no significant difference with the group “saline solution only” was observed, neither on a clinical point of view, nor on the EDX analysis. There were only slightly more important erosions in the Diphotérine[®] group, but the difference was not significant on a clinical point of view. Sodium and chloride did accumulate essentially in the superior layers of the cornea (fig 6/7).

Compared to the normal mineral composition of the rabbit cornea, sane or burnt (8 days after the burn), a loss of sulphur was detected for all irrigated groups (fig 8). The difference is highly significant for all groups. The low concentrations in sulphur are not limited to one part of the cornea but we can observe it on all layers of the cornea.

Discussion

The pH measures showed that a neutralisation was reached on the cornea surface after irrigation with a buffer solution Diphotérine[®]/Previn[®] or Isogutt[®]. The saline solution had a less important effect. No neutralisation was noticed in the aqueous humour with saline solution. The stroma was too resistant compared to the buffer power of the phosphate buffer or Diphotérine[®]. In other studies using a less important burn the buffer solution was able to decrease the pH to 7,4¹.

During the second phase, the effects of a long term irrigation with a buffer solution or an 0,9% saline solution were evaluated clinically and with an EDX analysis. The calcifications provoked by the phosphate buffer are due to an excess of calcium ions in the lachrymal liquid and of derived P ions from the buffer solution³. The mineralisation was favoured by the destroyed extra cellular matrix. The localisation in the superior layers of the cornea brings the proof of this pathogenese in the cornea : as the superior layers are in contact with the

lachrymal liquid and the buffer solution, they are the first to be calcified. Deeper calcifications could happen by diffusion of Ca and P ions.

The irrigation with an 0,9% saline solution induces an accumulation of sodium and chloride. Green⁵ described an alteration of the liaison sites of glycosaminoglycanes after digestion of the corneal stroma. These modified liaison sites are able to capture the sodium ions accumulated during repeated irrigations. The high concentrations in the epithelial layers show that a diffusion effect could be involved.

The sulphur decrease in all cornea layers examined clearly shows the negative influence of a prolonged irrigation with an inadequate solution. Figure 8 shows that in the burnt cornea of rabbit (8 days after the burn), without irrigation, the concentration in sulphur is not significantly different from the one in the sane corneas⁶. It is only after the first irrigation that the sulphur molecules were washed away from the cornea. It seems that a burn with an alkali provokes a chemical alteration of the sulphur liaison molecules like collagen or proteoglycanes. This alteration increases the sulphur loss after irrigation. But if the sulphur disappears then the aminoacids, the proteoglycanes and other components from this fundamental substance are also lost. Without sulphur and without the other fundamental molecules, no regeneration of the lamellar structure of the cornea is possible.

Conclusion

In this study, a very important trauma was inflicted to the rabbit eyes, which do not correspond to the burns observed in clinical practice. We did not expect any healing from the burnt corneas. It was however very easy to distinguish the negative influence of any prolonged irrigation. For the phosphate buffer group for example, the influence of phosphate on the naked cornea is visible through a complete calcification and a prolonged erosion. In the saline solution group, an influence on the mineral composition of the corneal stroma was proved by EDX analysis. A negative influence on the healing process is very probable in any case. In the presence of calcifications, there is no possible closure of the epithelium. The patient is blind. In all irrigation cases, the elimination of sulphur by washing impedes the healing of the fundamental substance of the corneal stroma, because sulphur is the principal component from glycosaminoglycanes and collagens⁷. The accumulation of ions like sodium or chloride induce an imbalance in the cornea hydration process. The reconstitution of glycosaminoglycanes and collagens will also be perturbed by this fact.

In case of blood replacement, the isotonicity of the substitute is indispensable for osmotic reasons. This has also to be true in the case of a naked cornea irrigation, as well as to accelerate the stroma deflation and to improve the healing process of the cornea. It is necessary to built an solution isotonic to the cornea or a healing solution containing the necessary elements and molecules for the stromal restructuration.

Until now, we never wonder about the long term use of a saline solution or a phosphate buffer, the last one being the base for many eye lotions. However many calcification cases were observed in Aachen after prolonged use of eye lotions based on phosphate buffer, particularly after ocular burns, which lead them to wonder if some solutions applied permanently on a naked corneal surface could not have an influence on the mineral composition and on the healing process of corneal injuries. It is necessary to mention that even in eye lotions, the buffer agent has to be adapted to the elementary needs of the cornea.

Legends (See the graphs on the original paper in French)

Fig 1 : Measures of the cornea surface pH after a 30 seconds burn with 1 M NaOH, followed by an irrigation during 5 minutes with 250ml of three different solutions. There are considerable differences in the pH reached. The saline solution, particularly, is absolutely not sufficient.

Fig 2 : Measures of the cornea surface pH after a 30 seconds burn with 1 M NaOH, followed by an irrigation during 5 minutes with 250ml of three different solutions. There are considerable differences in the pH reached. The saline solution, particularly, is absolutely not sufficient. There is a more important standard deviation with Diphotérine[®], which anyway reaches a good 9,5 pH.

Fig 3 : Calcification of the cornea (planimetric, in percentage of the total cornea surface). Isogutt provokes important calcifications. With Diphotérine[®]/Previn[®] some whittish surfaces appear, which could prove to be calcified.

Fig 4 : Elements of the cornea after a burn followed by a rinsing during 16 days. The phosphate buffer solution induces Phosphorus and Calcium deposits in the stroma of the burnt cornea.

Fig 5 : Distribution of Phosphorus on the cornea after a burn followed by a rinsing during 16 days. The phosphate buffer solution induces phosphorus deposits in all layers of the burnt cornea.

Fig 6 : Distribution of Sodium on the cornea after a burn followed by a rinsing during 16 days. The saline solution induces saline deposits in all layers of the burnt cornea with a gradient towards the endothelium.

Fig 7 : Distribution of Chloride on the cornea after a burn followed by a rinsing during 16 days. The gradient toward the endothelium is remarkable.

Fig 8 : Distribution of Sulphur on the cornea after a burn followed by a rinsing during 16 days. The loss in sulphur is considerable with all rinsing solutions.

References

See the reference on the original paper in French